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## Resistance of *Anopheles quadrimaculatus* Say Fourth-stage Larvae to Experimental Drought


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During recent years several investigators have noted the resistance of aquatic stages of mosquitoes to drought. According to available reports, only few such papers contain quantitative experimental data concerned with the genus *Anopheles*. Drought resistance of *A. walkeri* aquatic stages by Bick and Penn (1947), that of *A. quadrimaculatus* from preliminary experiments by Schoof *et. al.* (1945), and the results of an intensive study along these lines by Darrow (1949) on *A. quadrimaculatus*, are among the more pertinent publications. As early as 1899 Celli and Casagrandi, quoted by Stephens and Christophers (1908), reported that anopheline larvae were found to resist desiccation up to four days in moist soil. Subsequently, similar observations have been reported by Howard (1900), Nuttall and Shipley (1901), and Howard, Dyar, and Knab (1912). Nicholsky (1925) found that *A. maculatus* larvae could survive on moist soil for a period up to three days and Hinman ( 1938) noted the resistance of larval stages of *A. quadrimaculatus* **to** the drought caused by the draw-down of the Tennessee Valley Authority reservoirs. Hill and Cambournac (1941) found that larvae could survive up to twelve hours in the laboratory on soil with a moisture content of 20 per cent.

It seems worthwhile to consider further the resistance of these larvae under reproducible laboratory conditions since some ambiguity is necessarily associated with the terms "mud," "sand," "silt," "loam," and so forth. The


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ambiguity arises because the type of material contained in each is far from being standardized, and therefore the constituents vary greatly. The percentage of organic material in the natural substrata used throughout the work herein reported was determined experimentally with the hope of making these results reproducible in other laboratories.

## METHODS

Larvae of *A. quadrimaculatus* were obtained from the stock maintained at the Department of Tropical Medicine and Public Health of the Tulane University. Three types of substrata were employed for the exposure of fourth-stage larvae of *A. quadrimaculatus* to experimental drought. The first was called "river sand," the second, "river silt" and the third was filter paper. The river sand, collected from the banks of a nearby bayou, was composed almost entirely of sand *per se,* with only a small amount of extraneous material. Upon heating several dry uniform samples for periods of ten minutes over the hottest flame obtainable from a Bunsen burner, it was found to contain combustible organic material in the amount of 0.941 per cent. River silt, by comparison, had almost a fine loam composition and contained such organic material as small twigs, minor settled river debris, and a small percentage of decomposing foliage. By the same method of heating, the silt was found to contain 4.8 per cent organic material. The exposure on filter paper was made by cutting two pieces of Whatman No. 2 paper to fit each dish, as explained below. Filter paper substratum was used so that a comparison could be made between these results and those reported by Bick and Penn (1947) on *A. walkeri.*

The river sand and silt were placed in shallow dishes within an incubator set at 37° C. for a period of not less than two days. This brought about dehydration without appreciably changing the organic content. The organic portion, it was felt, might aid in the retention of moisture and thereby effect the survival of the larvae. After dehydration the *river sand* was added to Petri dishes in the amount of 20 gm. per dish and 10 ml. of tap water were added. The sand became completely moistened but no pools of surface water were visible. Fifteen early fourth instar larvae were added to each of several Petri dishes, half of which were covered with lids. The entire lot was placed in the insectary at a constant temperature of 79±1° F. Humidity readings in the insectary were recorded, the average relative humidity being 64 ±5 per cent. All dishes were protected from predators by the use of gauze netting. The larvae were examined at the termination of the various periods

of time by flooding with tap water and examining for motility during a period of time up to one hour. Each examined dish with its larvae was subsequently discarded. The *river silt* was weighed in amounts of 55 gm. per Petri dish, 15 ml. of tap water were added along with 20 early fourth instar larvae. Subsequent experimental conditions and examinations were the same as those used for river sand. Three and one-half ml. of tap water were added to each dish which contained two pieces of Whatman No. 2 filter paper cut to size. This amount of water thoroughly moistened the paper without giving rise to free surface water. Fifteen early fourth instar larvae were added to each dish and the same conditions and methods were followed as with the preceding substrata. The conditions then for each dish were as indicated in Table I.

TABLE I

| Substratum | Amount of substratum per dish | Amount of water per dish | Number of 4th stage larvae | Ml. water per gram substratum | Total no. of larvae examined |
|---|---|---|---|---|---|
| River sand | 20 gm. | 10 ml. | 15 | 0.50 | 720 |
| River silt | 55 gm. | 15 ml. | 20 | 0.27 | 1,020 |
| Filter paper | 2 pieces cut to size | 3.5 ml. | 15 | - | 270 |

## RESULTS

*River sand.* A total of 720 early fourth stage larvae were exposed to experimental drought on river sand, initially with 0.5 ml. of water per gram of sand. The results of subsequent examinations have been recorded in Table II. One half of the dishes were exposed to the atmospheric conditions of the room; the remainder were covered with Petri dish covers. Twenty-four hours after initiation of the drought an examination of 90 larvae in closed dishes revealed a 59.7 per cent average survival with 3.6 per cent of the water having evaporated. The 90 larvae examined in open dishes showed an average survival of 47.8 per cent with 73 per cent of the water having evaporated.

At the end of 48 hours the average survival of the 90 larvae in closed dishes was 37.5 per cent and an average of 5.2 per cent of the water had evaporated. At this time interval, 3.3 per cent of the 90 larvae in open dishes survived and 96 per cent of the water had evaporated from these dishes.

By the end of 72 hours of exposure to drought, 4.4 per cent of the 90 larvae examined in closed dishes had survived and 11.2 per cent of the initial water had evaporated. None of the larvae in the open dishes survived 72 hours of drought, 99 per cent of the water having evaporated.

TABLE II

**River Sand**

| Dishes open or closed | Av. relative room humidity (per cent) | Hours of exposure to drought | No. of larvae exposed | No. of larvae surviving | Per cent of survival | Av. amt. water per dish at given time (ml.) | Av. per cent of water remaining at given time | Av. amt. of water per gm. of sand at given time (ml.) |
|---|---|---|---|---|---|---|---|---|
| Closed | — | 0 | — | — | 100.0 | 10.00 | 100.0 | 0.50 |
| Closed | 65.5 | 24 | 90 | 55 | 59.7 | 9.64 | 96.4 | 0.48 |
| Closed | 62.4 | 48 | 90 | 24 | 37.6 | 9.48 | 94.8 | 0.47 |
| Closed | 61.0 | 72 | 90 | 4 | 4.4 | 8.88 | 88.8 | 0.44 |
| Closed | 63.2 | 96 | 90 | 0 | 0.0 | 8.50 | 85.0 | 0.42 |
| Open | — | 0 | — | — | 100.0 | 10.00 | 100.0 | 0.50 |
| Open | 59.2 | 24 | 90 | 43 | 47.8 | 2.70 | 27.0 | 0.13 |
| Open | 63.1 | 48 | 90 | 3 | 3.3 | 0.40 | 4.0 | 0.02 |
| Open | 62.7 | 72 | 90 | 0 | 0.0 | 0.10 | 1.0 | 0.005 |
| Open | 64.0 | 96 | 90 | 0 | 0.0 | 0.00 | 0.0 | 0.00 |

The final examination was made 96 hours after onset of drought. Among the 90 larvae examined in each of the closed and open dishes, none were found to have survived. Fifteen per cent of the water in the closed dishes had evaporated and 100 per cent of the original water in the open dishes had evaporated.

It was observed in later hours of exposure in closed dishes and throughout all observations made in open dishes that the sand particles were closely adhered to the mucoid covering of the larvae. Upon subsequent flooding these sand particles were removed from the larvae only with great difficulty. Several larvae which were found to be alive with such a covering were unable to rise to the water surface because of this sand which appeared to be glued particularly around the 9th abdominal segment. Only one of the 720 larvae examined was found to be pupating. It had died prior to complete pupation.

*River silt.* The results of resistance of early fourth instar larvae to drought on river silt are given in Table III. At the initiation of drought there was 0.27 ml. of water per gm. of silt. At the end of 24 hours exposure, ' the 120 larvae examined in closed dishes showed an average survival of 61.1 per cent and during this period of time 2 per cent of the original water had evaporated. At the same time interval, 35.5 per cent of the 120 larvae in open dishes survived with a corresponding loss of 48.4 per cent of the original water.

At the 48 hours interval an average of 30.9 per cent of the 120 larvae examined in closed dishes had survived and 3.7 per cent of the water had evaporated. In the open dishes out of 120 larvae examined an average of 2.5 per cent survived and 98.2 per cent of the water had evaporated.

Seventy-two hours after onset of drought, 9.5 per cent of the 120 larvae in closed dishes had survived with a corresponding water loss of 6.4 per cent. None of the 120 larvae in open dishes survived 72 hours of drought and 100 per cent of the water had evaporated from these open dishes.

The examination made 96 hours after drought showed that out of 120 larvae in closed dishes, an average of 5 per cent had survived with a 6.4 per cent loss of water. Again none of the 100 larvae examined in open dishes survived and none of the original water was left in these open dishes.

The final examination was made at the end of 120 hours of continuous drought. None of the 80 larvae examined in closed dishes were found to have survived, and the water loss at 120 hours was 7.3 per cent.

In five instances out of the 1,020 larvae examined, pupation had partially taken place with subsequent death. One larva had completely pupated and was living upon examination at the 24 hours interval, subsequently dying.

TABLE III

**River Silt**

| Dishes open or closed | Av. relative room humidity (per cent) | Hours of exposure to drought | No. of larvae exposed | No. of larvae surviving | Per cent of survival | Av. amt. water per dish at given time (ml.) | Av. per cent of water remaining at given time | Av. amt. of water per gm. of silt at given time (ml.) |
|---|---|---|---|---|---|---|---|---|
| Closed | — | 0 | — | — | 100.0 | 15.0 | 100.0 | 0.270 |
| Closed | 61.6 | 24 | 120 | 73 | 61.6 | 14.7 | 98.0 | 0.267 |
| Closed | 63.5 | 48 | 120 | 37 | 30.9 | 14.4 | 96.3 | 0.262 |
| Closed | 64.0 | 72 | 120 | 11 | 9.5 | 14.2 | 94.6 | 0.258 |
| Closed | 61.0 | 92 | 120 | 5 | 5.0 | 14.0 | 93.6 | 0.255 |
| Closed | 62.1 | 120 | 80 | 0 | 0.0 | 13.7 | 91.3 | 0.248 |
| Open | — | 0 | — | — | 100.0 | 15.0 | 100.0 | 0.270 |
| Open | 60.5 | 24 | 120 | 44 | 35.5 | 7.8 | 51.6 | 0.140 |
| Open | 59.7 | 48 | 120 | 3 | 2.5 | 0.2 | 1.8 | 0.003 |
| Open | 64.0 | 72 | 120 | 0 | 0.0 | 0.0 | 0.0 | 0.000 |
| Open | 67.5 | 96 | 100 | 0 | 0.0 | 0.0 | 0.0 | 0.000 |

The silt, unlike the sand, did not adhere as much to the bodies of the larvae although in about 10 per cent of the cases in closed dishes some form of fungus was found to be present on the larvae, apparently `sewing" them to the silt. Upon flooding and liberation from these fungus strands, many of the larvae became immediately active. Fungus formation in the open dishes was found in only about 2 per cent of the cases, presumably because of the much faster rate of water evaporation.

*Filter paper.* The results of the resistance of early fourth instar larvae to drought on filter paper are given in Table IV. At the initiation of drought there were 3.5 ml. of water per Petri dish. Twenty-four hours after onset of drought, *42.2* per cent of the *45* larvae in the closed dishes had survived, and *12.9* per cent of the water had evaporated. At this time interval, 4.4 per cent of the *45* larvae in open dishes survived, and 87 per cent of the water in these open dishes had evaporated.

At the *48* hours interval, *6.6* per cent of the 45 larvae in closed dishes had survived with a corresponding water loss of *21.5* per cent. None of the *45* larvae in open dishes survived *48* hours, and *100* per cent of the water in these dishes had evaporated.

Rapid evaporation of water from the open dishes was indicated by the large number of larvae which died within *24* hours. *In* some cases at 24 hours and all cases subsequently in open dishes the dehydration was so complete that recognition of the larvae as such was difficult. A total of 5 (2.8 per cent) out of 180 larvae examined in closed dishes were either partially or completely pupated. None of the larvae in open dishes pupated.

*Controls.* A total of 100 early fourth instar larvae were kept *in* open Petri dishes filled with water and used as controls. Periodic examination *rev* ealed that at the end of *120* hours *99* per cent had survived and 14 per cent had pupated.

## DISCUSSION OF RESULTS

On the basis of *720* larvae subjected to continuous drought on river sand and examined each *24* hours thereafter, it was found that *72* hours was the maximum time for larval survival *(4.4* per cent). This survival pertained only to larvae in closed dishes. The maximum survival time in the open dishes was 48 hours (3.3 per cent). It has been reported that larvae of *Anopheles claviger* can survive only 35 minutes on a dry substratum (Kalandadze and Sagatelova, 1945) and it can be similarly assumed that *A. quadrimaculatus* larvae can withstand complete dryness for only a matter of minutes.

TABLE IV

**Filter Paper**

| Dishes open or closed | Average relative room humidity (per cent) | Hours of exposure to drought | No of larvae exposed | No. of larvae surviving | Per cent of survival | Av. amt. water per dish at given time (ml.) | Av. per cent of water remaining at given time (ml.) |
|---|---|---|---|---|---|---|---|
| Closed | — | 0 | — | — | 100.0 | 3.50 | 100.0 |
| Closed | 69.5 | 24 | 45 | 19 | 42.2 | 3.05 | 87.1 |
| Closed | 62.4 | 48 | 45 | 3 | 6.6 | 2.75 | 78.5 |
| Closed | 64.7 | 72 | 45 | 0 | 0.0 | 2.10 | 60.0 |
| Closed | 63.1 | 96 | 45 | 0 | 0.0 | 1.80 | 51.4 |
| Open | — | 0 | — | — | 100.0 | 3.50 | 100.0 |
| Open | 69.5 | 24 | 45 | 2 | 4.4 | 0.45 | 13.0 |
| Open | 62.4 | 48 | 45 | 0 | 0.0 | 0.00 | 0.0 |

While it is true that the rate of death is in proportion to the rate of evaporation, it can be seen from Tables II and III that in the closed dishes there was an ultimate complete mortality while a high percentage of the original water remained. Therefore, the loss of water *per se* should be considered as simply one of several factors influencing the larval resistance to drought. The maximum time of survival in the closed dishes containing silt was 96 hours (5 per cent), while in the open dishes the maximum survival was 48 hours (3 per cent). In both the silt and sand, water evaporation from the open dishes was vastly greater than from the closed dishes. It was felt that the closed dishes would correspond to a high atmospheric humidity, and that this condition would play an important part in the length of larval survival. The maximum survival period on filter paper was in the closed dishes at 48 hours (6.6 per cent), while in the open dishes 2 per cent survived 24 hours and none thereafter. Comparison of the results on filter paper with those recorded by Bick and Penn (1947) with *A. walkeri* larvae shows a noticeable difference. These authors reported that larvae subjected to continuous drought survived 120 hours (27 per cent). Several explanations for this difference in findings are possible. There may well be a natural difference in drought resistance between *A. walkeri* and *A. quadrimaculatus*. The larvae under consideration by the above authors were from wild adults whereas the larvae used herein were from a strain which has been in captivity for a number of years. These workers also used uncut filter paper, thus providing more material to retain the moisture, and used 5 larvae per Petri dish whereas 15 per dish were used for the work herein reported.

Although the object of this work was simply to determine under somewhat standardized conditions the maximum time which larvae of *A. quadrimaculatus* can withstand continuous drought, several other observations have been made. One of the factors observed (in addition to the evaporation of water) which apparently hastens death is the mold found in the dishes containing the high-organic-content river silt. Undoubtedly this fungus hastens the death of the larvae either by actually relying on the larvae for nutrition, or by simply sewing the larvae to the substratum in such a way that subsequent flooding will not permit them to rise normally to the surface. The sand particles became securely glued onto the mucous covering of the larvae exposed on river sand. They apparently clogged the air tube, and in many cases prevented the larvae from rising to the surface upon flooding because of the excessive weight. Natural predators, as reported by Darrow (1949) also are to be taken into consideration. As mentioned previously they were kept away from the larvae in the work herein reported.

## SUMMARY AND CONCLUSIONS

Early fourth-instar larvae of *Anopheles quadrimaculatus* were exposed to continuous drought on three types of substrata, river sand, river silt, and filter paper, all in Petri dishes. Half of the dishes were exposed to the insectary humidity, the remainder were closed with Petri dish covers. The maximum length of survival was ascertained by daily examinations after a known amount of water had been added to each dish and the percentage of organic content assayed in the laboratory. Dishes and larvae were discarded subsequent to examinations. Out of the larvae examined, maximum survival in river sand (organic content 0.941 per cent, and initially with 0.5 ml. water per gm. sand) was 72 hours in the closed dishes. Of 1,020 larvae examined on river silt (organic content 4.8 per cent and initially with 0.27 ml. water per gm. silt) the maximum period of survival was 96 hours in the closed dishes. The maximum period of survival out of 270 larvae examined on filter paper (initially with 3.5 ml. water per dish) was 48 hours in the closed dishes.

Several factors dealing with the length of drought resistance by the larvae were noted. Water evaporation was found to be of prime importance only when evaporation takes place at a rate fast enough to kill the larvae before other factors appear. Other known factors are mold in the soil, clinging of the substrate particles to the larvae, and tendency of the larvae to attempt pupation.

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